

## Light and Electron Microscopic Study of Thyroid Gland in the African Giant Rat, *Cricetomys gambianus*, Waterhouse

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**Abstract.-** The morphology of thyroid gland of African giant rat (*Cricetomys gambianus*), a wild cricetid rodent has been described. Histologically, the morphological components of the thyroid (follicular cells, C-cells, colloid and interstitial tissue) were similar to that of some rodents like mice, rats, hamsters and guinea pig. However, peripheral vacuoles were rare in the colloid. Most of the follicular epithelia were predominantly lined by cuboidal and columnar cells, while squamous cells were uncommon. The mean internal follicular diameter of the small, medium and large round follicles were  $62.4 \pm 0.7 \mu\text{m}$ ,  $130.5 \pm 0.8 \mu\text{m}$  and  $176.6 \pm 0.6 \mu\text{m}$ , respectively. The follicular epithelial height was  $3.8 \pm 0.06 \mu\text{m}$  in flat cells,  $6.5 \pm 0.04 \mu\text{m}$  in cuboidal and  $8.3 \pm 0.08 \mu\text{m}$  in columnar cells. Ultrastructurally, apical pseudopods and microvilli were commonly observed. Large intraepithelial capillaries were also encountered. Parafollicular were located basally in cluster of 2 or 3 cells. They contained abundant electron dense granules within highly elongated cytoplasmic processes. The morphological features suggest that the thyroid gland is very active in response to the feral habit of the rat and the harsh tropical environment.

**Key Words:** Giant rat, thyroid gland, ultrastructure, follicular cells

### INTRODUCTION

Thyroid is the largest and one of the phylogenetically oldest endocrine glands in vertebrate species (Dickhoff and Darling, 1983). The gland is unique among vertebrate endocrine glands in that it stores secretory product (thyroid hormones) extracellularly (Braverman and Cooper, 2012). The thyroid follicle is the functional histological unit of the thyroid gland. It is made up of three principal components; the lining follicular cells, the basal parafollicular cells and the luminal colloid. The follicular cells produce thyroid hormones (triiodothyronine, T<sub>3</sub> and tetraiodothyronine, T<sub>4</sub>) which have important effects on cell proliferation, differentiation and migration as well as general growth and metabolism of embryos (Krees *et al.*, 2009). The parafollicular cells produce mainly calcitonin that regulates calcium metabolism and it also produces few other regulatory peptides of the thyroid such as somatostatin, chromogranin A and neuron specific enolase (NSE) that are probably involved in intrathyroidal regulation of follicular cells (Ahren, 1991; Sawicki, 1995).

There are marked variations in the morphology of the thyroid gland in different vertebrates. Even within a vertebrate class, there may be slight variation (Dyce *et al.*, 2002). The organ generally exhibits similar follicular structure amongst animal species, although there are certain gross, histological and ultrastructural variations. The thyroid follicular cells show variations in structure according to the functional status of the gland during growth and in response to environmental influences. It is generally agreed that variations in the organelle content of the thyroid follicular cell reflect variations in hormone synthesis, secretion and absorption (Harrison and Young, 1970; Gorbman *et al.*, 1983).

The African giant rat (AGR) (*Cricetomys gambianus*) also known as Gambian rat is wild cricetid rodent with an average adult weight of 1.4 kg occurring in Africa, predominantly confined to moist savannah regions (Ajayi, 1974). It provides supplementary protein diet for rural dwellers. There has been a continuous effort to domesticate it in some parts of Nigeria. African giant rat has shown potential for use as laboratory animal (Dipeolu *et al.*, 1981) and has been demonstrated to be a good host for the laboratory passage of *Schistosoma mansoni* and *Trypanosoma evansi* (Lariviere and Buttner, 1961). This rodent has been used to detect tuberculosis and sniff-out land mines in

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Mozambique and Angola (Lindow, 2001).

In the last three decades, several studies that included morphological studies have been conducted in an attempt to understand its biology and domestication (Ajayi, 1974, 1977; Kokkin, 1981; Knight, 1984; Oke and Aire, 1990; Oke *et al.*, 1995; Kelani and Durotoye, 2002; Onyeanusi *et al.*, 2007; Igbokwe and Nwaogu, 2009; Madekurozuwa *et al.*, 2010). However, no aspect of the morphology of the endocrine system in AGR, particularly the thyroid gland has been considered in all the available reports.

The main objective of this study is to provide information on the histological and ultrastructural features of the thyroid gland of the adult male African giant rat that would complement other biological information already available. This information would be useful to captive breeders and researchers in the life sciences.

## MATERIALS AND METHODS

Eight adult male AGR over 8 months weighing between 1.0-1.5 kg were used in this study. They were captured alive from the wild environment with metal cages, fed with standard laboratory animal feed in addition to raw potatoes, groundnut, palm nuts and bread during an acclimatization period of two weeks, and water was provided *ad libitum*. They were killed by cervical dislocation and the thyroid lobes were dissected out after death. For electron microscopy, thyroid lobes were diced into small pieces, immersed in modified Karnovsky's fluid containing 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer (pH7.4), subsequently washed in the same buffer and post-fixed in 1% osmium tetroxide. They were dehydrated in increasing concentrations of ethanol, infiltrated with a transitional fluid, propylene oxide and embedded in epoxy resin (Embed 812) following the method of Ko (Yön) and Akbulut (2012). Semi-thin sections (0.5-1 $\mu$ m) cut with ultramicrotome were stained with 25% toluidine blue for light microscopy and sections photographed with Moticam digital camera 2.0 attached to a microscope. For electron microscopy, suitable areas of interest were chosen on the trimmed blocks and ultra-thin sections (60-80nm) were cut and

contrasted with uranyl acetate and lead citrate. Philips CM10 transmission electron microscope (Eindhoven, The Netherlands) operated at 80 Kv was used for ultrastructural observations. Electron microscopic images were captured with a digital camera, iTEM MegaView® (Olympus Soft Imaging Solutions, Munster, Germany) attached to a desktop computer. For histometry, the ocular micrometer gauge calibrated with stage micrometer was used to measure the epithelial cell height from about 200 follicles from each lobe in several semi-thin sections using X10 objective magnification of Motic B1 Series Microscope (Motic China). The internal follicular diameter of small, medium and large-sized round follicles was also measured on the major and minor axis at right angles to each other according to the methods of Rao-Rupanagudi *et al.* (1992). The recorded values were expressed as mean and standard error of mean (SEM) using the statistical Package for Social Science (SPSS) Version 16.

## RESULTS

Histological features showed a very thin connective tissue capsule that enclosed the thyroid gland. The parenchyma was poorly divided into indistinct lobules by connective tissue septa that emanated from the capsule. The parenchyma was a glandular tissue with colloid-filled follicles of variable sizes and in more or less organized masses, with intervening connective tissue comprising abundant vascular tissue and scant connective tissue. Follicles were generally round to oval in sections and irregularly-shaped follicles with papillary invaginations into the lumen were sometimes encountered. Several interfollicular vascular elements penetrated the follicular epithelium as intraepithelial capillaries. Some follicles were devoid of luminal colloid (Fig.1A). The large follicles were observed predominantly in the periphery, while small follicles intermingled with few large follicles were present in the inner part of the gland. Highly irregular follicles of various sizes were commonly seen. Peripheral colloid vacuoles were not encountered in any follicle in this rodent. Large follicles were mainly lined by cuboidal and squamous follicular cells and were filled with colloid, while in small and medium-sized follicles

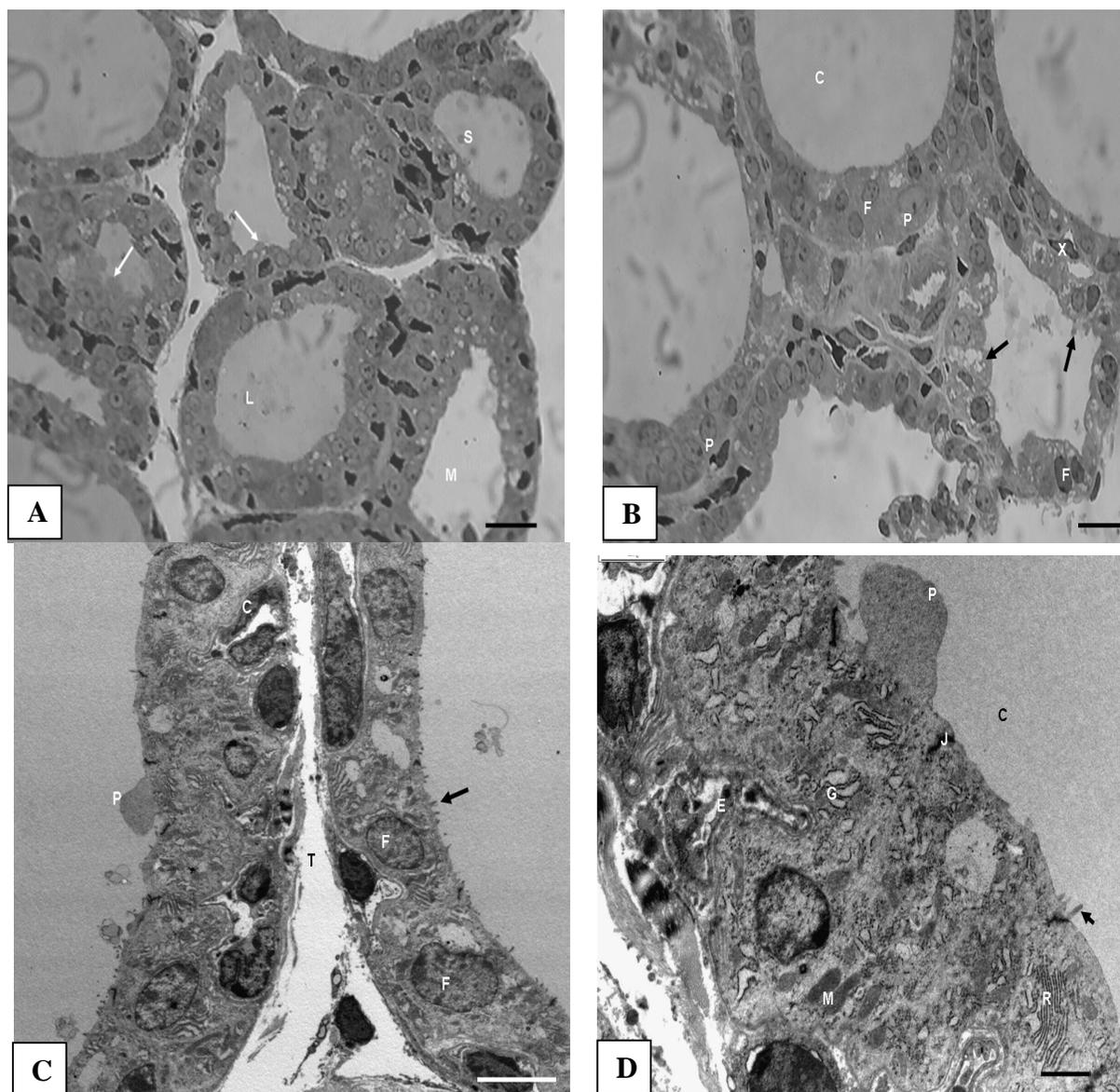


Fig. 1. Light micrographical (A,B) and electron micrographic structure (C, D) of thyroid gland of *Cricetomys gambianus*. A, shows follicle of various size, large (L), small (S). Note the absence of peripheral vacuoles in the follicles; B, shows numerous large distended follicles filled with colloid (C). Apical protrusions of the follicular epithelium are common (arrow). C, shows adjacent thyroid follicular epithelium showing follicular cells (F), Apical protrusions (P), Interfollicular connective tissue (T), Cilia (arrow) and interfollicular capillaries (C); D, shows thyroid follicular epithelium showing follicular cell with various features such as rough endoplasmic reticulum (R), Golgi body (G), mitochondria (M), junctional complex (J), apical protrusions (P), cilia (arrow head). Intraepithelial capillaries are apparent. Scale bar A, B 45  $\mu$ m; C, 10  $\mu$ m; D, 5  $\mu$ m.

columnar cells were quite common with cuboidal cells being occasionally present. Apical protrusions of the follicle with balloon-like form projecting into the follicular lumen were common. Few light

staining parafollicular cells were identified amongst follicular cells in the basal position and were rarely seen in the interfollicular position (Fig. 1B). The mean internal follicular diameter of the small,

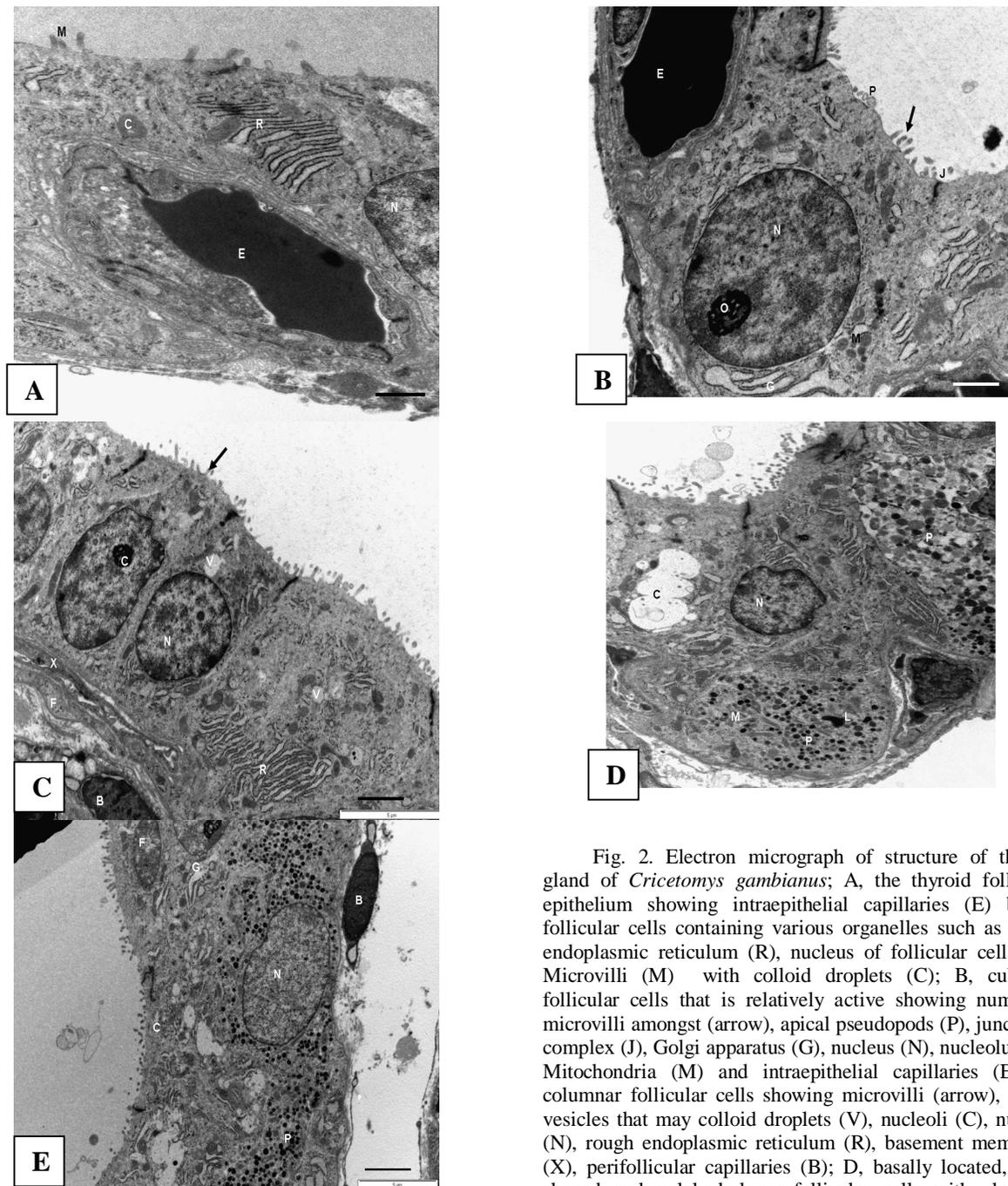


Fig. 2. Electron micrograph of structure of thyroid gland of *Cricetomys gambianus*; A, the thyroid follicular epithelium showing intraepithelial capillaries (E) beside follicular cells containing various organelles such as rough endoplasmic reticulum (R), nucleus of follicular cells (N), Microvilli (M) with colloid droplets (C); B, cuboidal follicular cells that is relatively active showing numerous microvilli amongst (arrow), apical pseudopods (P), junctional complex (J), Golgi apparatus (G), nucleus (N), nucleolus (O), Mitochondria (M) and intraepithelial capillaries (E); C, columnar follicular cells showing microvilli (arrow), apical vesicles that may colloid droplets (V), nucleoli (C), nucleus (N), rough endoplasmic reticulum (R), basement membrane (X), perifollicular capillaries (B); D, basally located, oval-shaped and polyhedral parafollicular cells with abundant cytoplasmic dense granules (P), clusters of mitochondria (M) and lysosomes are also present in the cytoplasm; E, parafollicular cells with elongated cytoplasmic process that contained numerous dense granules (P) amongst other cytoplasmic organelles. Note the thin rim of cytoplasm (C) of follicular cells separating it from the luminal colloid, nucleus of follicular cell (F) and fibroblasts (F) in the interfollicular connective tissue. Scale bar B, 2  $\mu\text{m}$ ; C, D, E, 5  $\mu\text{m}$

medium and large round follicles were  $62.4 \pm 0.7$ ,  $130.5 \pm 0.8$  and  $176.6 \pm 0.6 \mu\text{m}$  respectively. The follicular epithelial height was  $3.8 \pm 0.06 \mu\text{m}$  in flat (squamous) cells,  $6.5 \pm 0.04 \mu\text{m}$  in cuboidal and  $8.3 \pm 0.08 \mu\text{m}$  in columnar follicular cells.

Ultrastructurally, there were no remarkable differences between the organelle content of the cuboidal and columnar follicular cells. However, the columnar cells showed more irregularly arranged microvilli on the apical surfaces than the cuboidal and occasionally encountered squamous follicular cells. Microvilli were generally sparse in many follicular cells. Cilia that projected into colloid lumen were rarely seen and usually one cilium per cell was present. Apical protrusions with balloon-like shape and sometimes in form of pseudopods were observed on the surface of the follicular cells (Figs. 1C,D). The apical protrusions sometimes contained fine granular material mixed often with colloid. These pseudopods may be evidence of phagocytosis of colloid droplets by follicular cells. Further more, small 'pinocytic' invaginations of the apical plasma membranes were also evident. The junctional complexes between follicular cells in the apico-lateral borders were quite distinct with a prominent tight junction, from which single cilium sometimes projected from it into the luminal colloid (Fig. 1D). Beneath the junctional complex the lateral plasma membranes appeared to separate and formed well-marked intercellular channels. These channels contained few microvilli towards the base of the cell. Remarkably present beside the thyroid follicular cells were large intraepithelial capillaries, filled with electron dense blood cells (Figs. 2A,B). These capillaries filled with blood elements sometimes obscured the outline of endothelial cells in sections. The base of the follicular cells and the parafollicular cells rested on a distinct basement membrane. The perifollicular capillaries were abundant and clearly distinguished from the intraepithelial capillaries. It contained endothelial cells as well as luminal blood cells. The nuclei of follicular cells were generally centrally or basally located and varied in shape, but oval-shaped forms were commonly found in the cuboidal or columnar cells (Figs. 2B,C). Sometimes flattened nuclei were encountered in the few squamous cells present. The columnar follicular cells showed numerous

microvilli and with nucleolus in some nuclei, while in others it was missing. Fairly dilated cisternae of rough endoplasmic reticulum were distributed throughout the cytoplasm, particularly at the apical region of the cell. The Golgi apparatus was well developed and situated close to the nucleus and consisted of closely packed sacs and small vesicles. In most sections, the Golgi apparatus was located beneath the nucleus. The mitochondria were either round, rod-shaped or sometimes highly elongated and were distributed in the cytoplasm. In addition clusters of mitochondria of various shapes were seen in the basal and apical cytoplasm. Free ribosomes were very scant. Some cell inclusions that were not commonly observed in the follicular cell cytoplasm include; few small apical vesicles and typical large colloid droplets with similar electron density as the colloid. Small round dense secretory bodies presumed to be primary lysosomes were interspersed amongst profiles of mitochondria. Few degenerate follicular cells with pyknotic nuclei were sometimes encountered in the follicular epithelium.

Parafollicular cells of variable shape and size were identified by the basal location relative to the follicular cells. Occasionally, some were in the interfollicular position. They occurred singly and in group of 2 or 3 cells. They were predominantly round or ovoid-shaped, but triangular or spindle-shaped cells were sometimes encountered. Some of these cells possessed slender cytoplasmic process that made contact with adjacent cells. They lacked contact with the luminal colloid and were always separated from lumen by thin rim of cytoplasm of follicular cells (Fig. 2D). Parafollicular cells lacked complex interdigitations or specialized areas of plasma membranes with each other or with follicular cells. The most remarkable feature was the presence of several electron-dense secretory granules of variable size in the cytoplasm. They also spread along the elongated cytoplasmic process (Fig. 2E). The nuclei were generally ovoid and located towards one-end of the cells; centrally located nuclei equally apparent. Thin profiles of rough endoplasmic reticulum were beside the nucleus. Golgi apparatus was made up of several flattened saccules. In some plane of sections, the Golgi apparatus appeared elongated and arranged in

parallel stacks. Mitochondria with variable shape were distributed evenly in the cytoplasm and sometimes in clusters. Lysosome-like dense bodies were also observed in the cytoplasm.

### DISCUSSION

The overall histological and ultrastructural observations in the thyroid gland of adult African giant rat (AGR) showed some similarities as in mouse (Ekholm and Sjöstrand, 1957), rat (Wissig, 1960), hamster (Lietz and Böcker, 1974), rabbits (Parchami and Dehkodi, 2006) and several other mammals (Fujita, 1975, 1988 for reviews). These similarities relate to the shape of follicle, follicular diameter, follicular cell height, arrangement of small and large-sized follicles within the thyroid parenchyma, presence of colloid, intermingling of cuboidal, columnar and squamous follicular cells within a follicle and the presence of scanty developed connective tissue stroma, with copious vascular sinuses. These observations agreed with the much earlier histological observations of these features in rodents like rats, mice, rabbits, and hamsters (Yagizawa, 1956).

The ultrastructural features of the follicular cells showed some apical modifications of the cell, various cytoplasmic organelles, nucleus and other cell inclusions required for thyroid function as have been observed in thyroid of other mammals (Pantic, 1974). Some features of the thyroid in this presently study that was not commonly encountered in other thyroid of mammals were absence of peripheral colloid vacuoles, presence of intraepithelial capillaries, balloon-like apical protrusions suggesting high apocrine secretory activity in the follicular cells and presence of parafollicular cells with long slender cytoplasmic processes. These parafollicular cells with oval shape and some with long slender cytoplasmic processes were filled with characteristic cytoplasmic dense secretory granules presumed to be the source of calcitonin and other regulatory peptides as demonstrated in several mammals (Nunez and Geshon, 1978; Ahren, 1991).

Specifically, the absence of peripheral resorption vacuoles in the colloid under light microscope and its presence in few numbers as intracellular vacuoles (colloid droplets) within

follicular cells indicate reduced activity of the thyroid follicular cells of AGR. Lysosome-like bodies were also not commonly observed supporting the opinion that that the process of liberating thyroid hormones from thyroglobulin may be slow in the AGR follicular cells.

Pseudopods and protrusions with dome-shape or balloon-like shape were commonly observed on the apical surface. It contained fine granular matrix often with colloid droplet, this feature signify apocrine secretions as has been observed in camel (Atoji *et al.*, 1999) and nomadic reared White Fulani (zebu) cattle (Igbokwe, 2013). Pseudopod and apical blebs have been observed on apical surface of hyperplastic rat thyroid in vivo (Zeligs and Wollman, 1977). During severe stimulation of thyroid in some severe conditions, pseudopods are produced at the luminal surface of follicular cells facing the colloid and this phenomenon triggers endocytosis of stored thyroglobulin, resulting in large colloid droplets (Fujita, 1988). This feature observed in the present study could be as a result of the intense stimulation of the thyroid by the wild natural environment.

Intraepithelial capillaries were remarkably observed in the follicular epithelium adjacent to follicular cells. There were also perifollicular capillaries with fibroblasts and collagen fibrils within the interfollicular connective tissue. These enormous vascular elements suggest that the thyroid parenchyma is highly vascularised to receive large quantity of hormones into the general circulation. Intraepithelial capillaries were recognizable because more than 2/3 of their circumference is surrounded by follicular epithelium as previously shown by Lietz and Böcker (1974) and Sato (1959) in golden hamster. Intraepithelial capillaries have also been reported in the jimpy mice (Liu, 1984). They were characterized by endothelial cells with oval, irregularly contoured nuclei, dense heterochromatin and cytoplasm rich in organelles. Concerning the significance of intraepithelial capillaries in the thyroid of AGR, it may be that thyroid hormones of low molecular weight may enter the circulation at these capillaries as have been suggested by Lietz and Böcker (1974) in golden hamster. The perifollicular capillaries of the present study corresponded to that seen in most mammalian

thyroids that include the mouse (Ekholm and Sjöstrand, 1957), rat (Wissig, 1960), golden hamster (Lietz and Böcker, 1974). The existence of copious vascularisation of the thyroid with intraepithelial and perifollicular vascular elements in AGR may be an adaptation to the wild harsh environment in the sub-Saharan tropical climate.

Parafollicular cells are generally described as being oval or polyhedral in shape (Fujita, 1975). In the present study, we encountered oval cells and some with slender cytoplasmic processes filled with numerous dense secretory granules and these processes extended between neighbouring cells. Similar parafollicular cells with cytoplasmic processes have been observed in guinea-pigs, tree shrews and neonatal human beings (Roediger, 1973). Also the parafollicular cells in the rat and rabbit have been described as having slender cytoplasmic processes (Stux *et al.*, 1961). The existence of this modification of the cytoplasm with its numerous dense secretory granules and few lysosomes indicate that it may help to increase cytoplasmic area for the production of calcitonin and phagocytosis of cellular debris.

In conclusion, the morphologic features of the thyroid of AGR did not differ essentially from that of other mammals especially rodents and that it is moderately active in the transport, synthesis and release of thyroglobulin, and secretion of thyroid hormones. However the few uncommon features in the thyroid are probably due to species differences and in response of this rodent to several environmental and climatic effects.

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#### Formal statement

The authors wish to state that all procedures involving animals were carried out according to the guidelines for the protection of animal welfare in the

University of Nigeria Nsukka, Enugu State, Nigeria.

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